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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/549,389

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Shigeru Kanaoka

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EXAMINER

PANDE, SUCHIRA

ART UNIT

PAPER NUMBER

1637

MAIL DATE

DELIVERY MODE

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PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

<b>Office Action Summary</b>	<b>Application No.</b> 10/549,389	<b>Applicant(s)</b> KANAOKA, SHIGERU	
	<b>Examiner</b> SUCHIRA PANDE	<b>Art Unit</b> 1637	

**-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --**

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 27 April 2009.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 5, 15-18, 20, 22 and 23 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 5, 15-18, 20, 22 and 23 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)            | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)   | Paper No(s)/Mail Date. _____                                      |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>1/28/2008; 4/27/2009</u> .                                    | 6) <input type="checkbox"/> Other: _____                          |

## **DETAILED ACTION**

### ***Continued Examination Under 37 CFR 1.114***

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on April 27, 2009 has been entered.

### ***Claim Status***

2. Claims 1-4, 6-14, 19 and 21 are cancelled. Previously examined claims 5, 15-18, 20, 22 and 23 are pending in this application and will be examined in this action. No claims have been amended.

### ***Information Disclosure Statement***

3. The information disclosure statement (IDS) submitted on January 28, 2008 and April 27, 2009 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

### ***Response to Arguments re pending 103 rejections***

4. Declaration filed on April 27, 2009 is acknowledged. Applicant argued unexpected results in amendment filed in September 30, 2008 which was considered unpersuasive. Applicant has provided additional data to support their contention that their method shows unexpected improvement for detection of colon cancer using occult blood and CEA marker.

Art Unit: 1637

5. Examiner would like to point out that the additional data presented is found unpersuasive because the additional data presented is not commiserate with the scope of the claimed invention. Instant claims do not recite a particular level of sensitivity or specificity of detection of COX-2.

6. To clarify the record, Examiner would like to point out following facts taught to one of ordinary skill in the art by prior art, which make the invention recited in instant claims obvious.

7. Prior art Alexander and Raicht teaches use of human stool as starting material from which Total RNA is extracted (see title). They also teach stool, which picks up sloughed-off colonic epithelial cells in its passage through the colon, would be ideal for sampling colonic mucosa (see page 2652 par. 2). Hence the Total RNA extracted by Alexander and Raicht also contains RNA isolated from the colonic epithelial cells of the subject from which the stool was collected. This RNA is subjected to RT PCR to detect various genes. Alexander and Raicht do not teach detection of COX-2.

8. Shattuck-Brandt et al. (1999) Molecular Carcinogenesis vol. 24: pp177-187 teaches to one of ordinary skill that COX-2 expression is markedly increased in 85-90% of human colorectal adenocarcinomas, whereas COX-1 levels remain unchanged (see page 178 par. 2). They state "In human cancers----- whereas COX-2 was expressed throughout the neoplastic epithelium, with the strongest expression in well – differentiated regions. On the basis of these results, one could speculate that the increase in COX-2 expression occurs very early" (See page 184 par. 1) They also teach detection of COX-2 by RT PCR. Thus Shattuck-Brandt et al. teach to one of ordinary

Art Unit: 1637

skill that COX-2 expression is associated with colon cancers and COX-2 is expressed in the epithelial cells of the cancerous tissue.

**9.** Lagerholm et al. (April 2001) Gastroenterology volume 120 No. 5 supplement 1 Abstract no 16 in page A4 teach collection of stool samples from patients suffering with inflammatory Bowel Disease (IBD) and healthy control subjects. They used RT PCR to detect presence of COX-2. Colonocytes present in stool of human subjects were the source of RNA used for RT PCR detection. Marked COX-2 expression was detected in 100% of patients suffering with IBD while no expression of COX-2 was seen in healthy control subjects. In all subjects, the presence of epithelial cells was confirmed by the demonstrated expression of cytokeratin -19. Thus Lagerholm et al. teach to one of ordinary skill that stool of humans contains epithelial cells from which RNA can be isolated and if these epithelial cells are expressing COX-2 then RT-PCR will be able to detect its presence.

**10.** Based on the combined teachings of prior art cited above, It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made that the RNA made by method of Alexander and Raicht contains RNA that is derived from epithelial cells that were present in human stool. If the subject was suffering from colon cancer then the epithelial cells present in his stool would be expressing COX-2 whereas if the subject was healthy control then the epithelial cells present in his stool would not be expressing COX-2. Hence one of ordinary skill in the art has a reasonable expectation to be able to detect COX-2 using RT PCR from RNA samples obtained from stool of colon cancer patients and no detection of COX-2 from RNA samples obtained

Art Unit: 1637

from stool of healthy subjects (controls). In controls one of ordinary skill in the art will expect to see expression of control genes such as cytokeratin -19 that is expressed in epithelial cells.

Hence in view of Examiner the invention recited in claim 5 of instant application is obvious to one of ordinary skill in view of the teachings cited above. Examiner has used new art to explain the above logic that was not cited before. Examiner has withdrawn all previously cited 103 rejections, and rejecting the pending claims over new art.

***Claim Rejections - 35 USC § 102***

11. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

12. Claims 5, 17-18, 20, 22 and 23 are rejected under 35 U.S.C. 102(b) as being anticipated by Chapkin et al. (US pat. 6,258,541 B1) as evidenced by Product review of Ambion's Poly A+ Pure kit.

Regarding claim 5, Chapkin et al. teach COX-2 (see col. 6 line 14 where COX-2 referred as cyclooxygenase II is taught) detecting method (see col. 5 lines 64-65 where detection of predictive risk marker of colon cancer is taught. One of the biomarkers recited is COX-2) for detecting colon cancer (see abstract) comprising:

a) homogenizing collected feces in the presence of an RNase inhibitor to prepare a suspension thereof, without separating cell components from the feces (see col. 5 lines 8-10 where direct isolation of RNA from feces is taught. Also see col. 7

Art Unit: 1637

Experimental methods Isolation of poly A+ RNA from feces step 1. Where homogenization of feces with a pestle in lysis buffer of Ambion poly A+ Pure kit is taught. Thus teaching homogenizing collected feces in the presence of an RNase inhibitor to prepare a suspension thereof, without separating cell components from the feces)

b) extracting RNA from the suspension from step a) to provide extracted RNA (see col. 7 steps 2-10 which result in extracted RNA starting from step a). This is poly A+ RNA);

c) carrying out reverse transcription on the extracted RNA from step b) to provide cDNA (see col. 10 lines 31-32 where RT PCR is taught. Also see col. 10 lines 34-35 where cDNA production using reverse transcriptase is taught. Thus teaching carrying out reverse transcription on the extracted RNA from step b) to provide cDNA);

d) amplifying the cDNA from step c) (see col. 10 line 37 where PCR of the cDNA made is taught); and

e) detecting the amplified COX-2 from step d) (see col. 11 lines 23-26 where running PCR products on gels, staining them by ethidium bromide and scanning them to determine intensity is taught. Thus teaching detecting the amplified COX-2 from step d)).

Regarding claims 17 and 20, Chapkin et al. teach wherein the feces is frozen (see col. 9 line 3 where freezing of feces samples at -80°C is taught.

Regarding claims 18 and 20, Chapkin et al. teach wherein the RNase inhibition is selected from the group consisting of (i) guanidine thiocyanate, (ii) a homogenous liquid

Art Unit: 1637

containing phenol and guanidine thiocyanate and (iii) a 14M solution of guanidine salts, urea and a RNA binding resin (see col. 9 lines 1-2 where lysis solution from Poly A+ Pure kit, Ambion is taught. By teaching lysis solution from Poly A+ Pure kit, Chapkin et al. teach wherein the RNase inhibition is selected from the group consisting of (i) guanidine thiocyanate. See product review provided by Examiner).

Regarding claims 22 and 23, Chapkin et al. teach wherein the feces is human feces. (see col. 7 line 23 where human feces is taught)

Thus claims 5, 17-18, 20, 22 and 23 are anticipated by Chapkin et al.

***Claim Rejections - 35 USC § 103***

13. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

14. Claims 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chapkin et al. as applied to claim 5 above further in view of further in view of Godfrey et al. (US Pat. 7101663 B2 issued September 5, 2006 filed on March 4, 2002—previously cited).

Regarding claim 15 Chapkin et al. teach the method of claim 5 and teach RT PCR. But Chapkin et al. do not teach wherein



Art Unit: 1637

in step e) amplifying the cDNA from step d) is carried out by a nested PCR.

Regarding claim 15, Godfrey et al. teach in step e) amplifying the cDNA from step d) is carried out by a nested PCR (see col 15 line 61 where nested PCR is taught).

Regarding claim 16, Godfrey et al. teach, wherein the amplification is carried out by a PCR and a first round of the PCR is executed for 20 cycles (see col. 20 lines 19-20 where Godfrey et al. teach PCR is carried out in two 20-cycle steps. Thus Godfrey et al. teach wherein the amplification is carried out by a PCR and a first round of the PCR is executed for 20 cycles).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Godfrey et al. in the method of Chapkin et al.

The motivation to do so is provided to one of ordinary skill in the art by Godfrey et al. who state "Quantitative RT-PCR is a sensitive technique and is particularly useful for the analysis of samples containing limited amounts of nucleic acids, such as in clinical tissues-----". When quantitating these small amounts of RNA and/or very low abundance mRNA species, obtaining maximum sensitivity from a quantitative RT-PCR is extremely important. While consecutive rounds of nested PCR are often used to obtain maximum sensitivity, this is difficult to achieve and still maintain accurate quantitation.

Furthermore, multiple rounds of PCR increase the risk of contamination, a serious problem when working at desired sensitivity levels. One tube RT-PCR reduces the risk of contamination -----because the reaction tubes are never opened. Theoretically, a one tube procedure should have the same sensitivity as a two step approach (separate

Art Unit: 1637

RT followed by PCR) but in practice this is not the case". (see col. 15 lines 28-43). They go on to list out the reasons why this is the case. Finally they state "In a two –step or **nested RT-PCR procedure**, specificity can be achieved with the use of hot-start PCR and a primer set 5' upstream from the RT primer. However, this is not the case in a one –tube procedure unless one is willing to open the reaction tube to add new primers (thus making it a one –tube but two step procedure). It has been hypothesized that by using an external RT primer and keeping the RT and PCR primers separated during the RT step, PCR specificity and therefore sensitivity in a one –tube RT-PCR should be maintainable-----Here, a modified one –tube RT-PCR assay that greatly increases sensitivity and can be used for quantitative RT-PCR----is presented." (see col 15 lines 61- col. 16 line 5). Thus explicitly teaching to one of ordinary skill that by using this modified method one can perform **nested PCR** in one tube closed format and at the same time have a sensitive quantitative RT-PCR.

15. Claims 5, 17-18, 20 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Alexander & Raicht (1998) Digestive Diseases and Sciences Vol. 43 No. 12 pp 2652-2658 (previously cited) as evidenced by Ultraspec™-II RNA isolation system Biotecx Bulletin No. 28, 1993 in view of Shattuck-Brandt et al. (1999) Molecular Carcinogenesis vol. 24: pp177-187 (provided by Applicant in IDS) and Lagerholm et al. (April 2001) Gastroenterology volume 120 No. 5 supplement 1 Abstract no 16 in page A4 (newly cited).

Art Unit: 1637

Regarding claim 5, Alexander and Raicht teach a method for detecting colon cancer (See page 2652 par.1-2, where colon neoplasia and methods of diagnosing it are taught) comprising:

a) homogenizing collected feces (human stool) in the presence of an RNase inhibitor(Ultraspec II reagent from Biotecx Laboratories contains chaotropic agent 14 M guanidium salt that are potent inhibitors of RNase) to prepare a suspension thereof (See page 2653 Materials and Methods par. 2-4 under Purification of total RNA from stool samples), without separating cell components from the feces (the method taught by Alexander and Raicht directly homogenizes the stool without separating cell components see page 2653 where frozen piece of stool is made into a slurry) ;

b) extracting RNA from the suspension from step a) to provide extracted RNA (see page 2653 section purification of RNA);

c) carrying out reverse transcription on the extracted RNA from step b) to provide cDNA (see page 2654 par. 3-4 where RT-PCR is taught);

d) amplifying the cDNA from step c) (see page 2654 par. 5-6 where PCR amplification of cDNA is taught); and

e) detecting the amplified tumor marker from step d) wherein the tumor marker is thereby detected (see page 2654 par. 7 and Results par. 3 where detection of amplified cDNA by gel electrophoresis is taught. Thus teaching detection of selected marker).

Regarding claim 17 Alexander and Raicht teach wherein the feces is frozen (see page 2653 par. 2 under Purification of Total RNA from Stool Samples, where freezing for Stool sample in Liquid Nitrogen is taught).

Art Unit: 1637

Regarding claim 18 Alexander and Raicht teach wherein the RNase inhibition is selected from the group consisting of (i) guanidine thiocyanate, (ii) a homogenous liquid containing phenol and guanidine thiocyanate and (iii) a 14M solution of guanidine salts, urea and a RNA binding resin (Alexander and Raicht teach use of Ultraspec II reagent, a single step RNA purification from Biotecx Laboratories. This reagent contains 14 M solution of guanidine salts. The formulation is based on a method of Chomczynski and Sacchi that uses guanidinium thiocyanate-phenol-chloroform for RNA isolation. See Biotecx Bulletin No:28, 1993, Introduction and Reference no 3.).

Regarding claim 20 Alexander and Raicht teach wherein the feces is frozen (see page 2653 par. 2 under Purification of Total RNA from Stool Samples, where freezing for Stool sample in Liquid Nitrogen is taught).;

and the RNase inhibitor is guanidine thiocyanate (Alexander and Raicht teach use of Ultraspec II reagent, a single step RNA purification from Biotecx Laboratories. This reagent contains 14 M solution of guanidine salts. The formulation is based on a method of Chomczynski and Sacchi that uses guanidinium thiocyanate-phenol-chloroform for RNA isolation. See Biotecx Bulletin No:28, 1993, Introduction and Reference no 3.).

Regarding claims 22 and 23 Alexander and Raicht teach wherein the feces is human feces (see title where human stool is taught).

Regarding claim 5 Alexander and Raicht do not teach use of the marker COX-2 as a marker suitable for colon cancer detection.

Regarding claim 5, Shattuck-Brandt et al. teaches the tumor marker COX-2 is expressed in human colon cancer (see title and abstract where COX-2, a colon cancer marker is taught).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use COX-2 tumor marker taught by Shattuck-Brandt et al. in the method of Alexander and Raicht for diagnosing colon cancer. The motivation to do so is provided to one of ordinary skill by teachings of Alexander & Raicht; Shattuck-Brandt et al. and Lagerholm et al.

Alexander & Raicht teaches use of human stool as starting material from which Total RNA is extracted (see title). They also teach stool, which picks up sloughed-off colonic epithelial cells in its passage through the colon, would be ideal for sampling colonic mucosa (see page 2652 par. 2). Hence the Total RNA extracted by Alexander & Raicht also contains RNA isolated from the colonic epithelial cells of the subject from which the stool was collected. This RNA is subjected to RT PCR to detect various genes.

Shattuck-Brandt et al. teaches to one of ordinary skill that COX-2 expression is markedly increased in 85-90% of human colorectal adenocarcinomas, whereas COX-1 levels remain unchanged (see page 178 par. 2). They state "In human cancers-----  
whereas COX-2 was expressed throughout the neoplastic epithelium, with the strongest expression in well -differentiated regions. On the basis of these results, one could speculate that the increase in COX-2 expression occurs very early (See page 184 par. 1)." They also teach detection of COX-2 by RT PCR (see page 179 section RT-PCR).

Art Unit: 1637

Thus Shattuck-Brandt et al. teach to one of ordinary skill that COX-2 expression is associated with colon cancers and COX-2 is expressed in the epithelial cells of the cancerous tissue.

Lagerholm et al. teach collection of stool samples from patients suffering with inflammatory Bowel Disease (IBD) and healthy control subjects. They used RT PCR to detect presence of COX-2. Colonocytes present in stool of human subjects were the source of RNA used for RT PCR detection. Marked COX-2 expression was detected in 100% of patients suffering with IBD while no expression of COX-2 was seen in healthy control subjects. In all subjects, the presence of epithelial cells was confirmed by the demonstrated expression of cytokeratin -19. Thus Lagerholm et al. teach to one of ordinary skill that stool of humans contains epithelial cells from which RNA can be isolated and if these epithelial cells are expressing COX-2 then RT-PCR will be able to detect its presence.

Based on the combined teachings of prior art cited above, It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made that the RNA made by method of Alexander & Raicht contains RNA that is derived from epithelial cells that were present in human stool. If the subject was suffering from colon cancer then the epithelial cells present in his stool would be expressing COX-2 whereas if the subject was healthy control then the epithelial cells present in his stool would not be expressing COX-2. Hence one of ordinary skill in the art has a reasonable expectation to be able to detect COX-2 using RT PCR from RNA samples obtained from stool of colon cancer patients and no detection of COX-2 from RNA samples obtained

Art Unit: 1637

from stool of healthy subjects (controls). In controls one of ordinary skill in the art will expect to see expression of control genes such as cytokeratin -19 that is expressed in epithelial cells.

16. Claims 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Alexander & Raicht ; Shattuck-Brandt et al. and Lagerholm et al. as applied to claim 5 above further in view of Godfrey et al. (US Pat. 7101663 B2 issued September 5, 2006 filed on March 4, 2002—previously cited).

Regarding claim 15 Alexander & Raicht; Shattuck-Brandt et al. and Lagerholm et al. teach the method of claim 5 and teach RT PCR. But Alexander & Raicht; Shattuck-Brandt et al. and Lagerholm et al. do not teach wherein

in step e) amplifying the cDNA from step d) is carried out by a nested PCR.

Regarding claim 15, Godfrey et al. teach in step e) amplifying the cDNA from step d) is carried out by a nested PCR (see col 15 line 61 where nested PCR is taught).

Regarding claim 16, Godfrey et al. teach, wherein the amplification is carried out by a PCR and a first round of the PCR is executed for 20 cycles (see col. 20 lines 19-20 where Godfrey et al. teach PCR is carried out in two 20-cycle steps. Thus Godfrey et al. teach wherein the amplification is carried out by a PCR and a first round of the PCR is executed for 20 cycles).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Godfrey et al. in the method of Alexander & Raicht; Shattuck-Brandt et al. and Lagerholm et al.

Art Unit: 1637

The motivation to do so is provided to one of ordinary skill in the art by Godfrey et al. who state “ Quantitative RT-PCR is a sensitive technique and is particularly useful for the analysis of samples containing limited amounts of nuclei acids, such as in clinical tissues----- . When quantitating these small amounts of RNA and/or very low abundance mRNA species, obtaining maximum sensitivity from a quantitative RT-PCR is extremely important. While consecutive rounds of nested PCR are often used to obtain maximum sensitivity, this is difficult to achieve and still maintain accurate quantitation. Furthermore, multiple rounds of PCR increase the risk of contamination, a serious problem when working at desired sensitivity levels. One tube RT-PCR reduces the risk of contamination -----because the reaction tubes are never opened. Theoretically, a one tube procedure should have the same sensitivity as a two step approach (separate RT followed by PCR) but in practice this is not the case”. (see col. 15 lines 28-43). They go on to list out the reasons why this is the case. Finally they state “In a two –step or **nested RT-PCR procedure**, specificity can be achieved with the use if hot-start PCR and a primer set 5’ upstream from the RT primer. However, this is not the case in a one –tube procedure unless one is willing to open the reaction tube to add new primers (thus making it a one –tube but two step procedure). It has been hypothesized that by using an external RT primer and keeping the RT and PCR primers separated during the RT step, PCR specificity and therefore sensitivity in a one –tube RT-PCR should be maintainable-----Here, a modified one –tube RT-PCR assay that greatly increases sensitivity and can be used for quantitative RT-PCR----is presented.” (see col 15 lines 61- col. 16 line 5). Thus explicitly teaching to one of ordinary skill that by using this



Art Unit: 1637

modified method one can perform ***nested PCR*** in one tube closed format and at the same time have a sensitive quantitative RT-PCR.

### ***Conclusion***

17. All claims under consideration 5, 15-18, 20 and 22-23 are rejected over prior art.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Suchira Pande

Application/Control Number: 10/549,389  
Art Unit: 1637

Page 17

Examiner  
Art Unit 1637

/Teresa E Strzelecka/

Primary Examiner, Art Unit 1637

May 21, 2009